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Effects of Eggshell Coloration on Egg Cannibalism among Glaucous-winged Gulls

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Abstract

A common source of reproductive loss in gulls is egg cannibalism. At a large Glaucous-winged Gull (*Larus glaucescens)* colony on Protection Island, Washington, cannibalism accounts for 55% of egg loss. Because cannibalism is a form of predation and birds have a highly developed sense of vision, I hypothesized that visible light coloration of Glaucous-winged Gull eggs plays a role in determining whether they are cannibalized. I used logistic regression to test whether egg fate was related to egg brightness, specific coloration, specific spot coverage, coloration relative to the most common coloration, and spot coverage relative to the most common spot coverage. The odds that an egg was cannibalized increased when an egg was more intensely red, more intensely green, when its green value was closer to the most common green value, and when its combined color value was closer to the most common combined color value. These results suggest that cannibals may prefer eggs with the most common coloration.

Key words: bird vision, egg cannibalism, egg color, Glaucous-winged Gull, ImageJ

Introduction

When sea surface temperatures increase in the Salish Sea during El Niño events, cool water levels drop in the water column, as do levels of plankton and feeder fish. Because gulls are surface-feeders and not diving birds, the movement of fish to deeper water makes it difficult for gulls to procure enough food during their annual breeding season. At a large Glaucous-winged Gull (*Larus glaucescens*) colony on Protection Island, Washington, cannibalism accounts for 55% of egg loss. Previous studies suggest cannibalism may function as a life-boat mechanism during years with low resource levels (Henson 1997, Hayward et al. 2014).

Although the majority of research regarding egg cannibalism in gulls centers on factors influencing rates of cannibalism (Brower et al. 1994, Good 2002, Davis et al. 1974, Polski et al. in submission), no study has investigated the factors used by cannibalistic gulls to decide whether to predate one egg over another. Because cannibalism is a form of predation and birds have a highly developed sense of vision, one might hypothesize that visual factors influencing predation should also play a role in the decision-making of cannibals (Odeen et al. 2013). Indeed, Yang et al. (2015) discovered that ultraviolet (UV)-blocked pigeon eggs were less likely to be eaten by aerial predators than non-UV-blocked eggs. They suggested that the birds' sensitivity to UV light might play a role in detecting and consuming prey eggs. Like pigeons, gulls can see both UV light and the visible spectrum detectable by humans (Bennet et al. 2015). A study conducted by Smith showed that Glaucous-winged Gulls preferentially predate UV-blocked chicken eggs rather than control chicken eggs (Smith et al. 2017). Given that gull eggs reflect less UV light than chicken eggs (J. L. Hayward, personal communication), gulls predated chicken eggs that were most similar to their own.

In this study, I tested the hypothesis that there is a relationship between egg cannibalism and the visual appearance of an egg. Due to the absence of UV images, I only investigated the visible light coloration of eggshells in relation to egg cannibalism. I tested six subhypotheses:

Subhypothesis A: Cannibalistic gulls select eggs based on the degree of brightness of the egg.

Subhypothesis B: Cannibalistic gulls select eggs based on the absolute difference between the egg's brightness and the most common egg brightness in the population.

Subhypothesis C: Cannibalistic gulls select eggs based on the degree of color intensity of the egg.

Subhypothesis D: Cannibalistic gulls select eggs based on the absolute difference between the egg coloration and the most common egg coloration in the population.

Subhypothesis E: Cannibalistic gulls select eggs based on degree of spot coverage.

Subhypothesis F: Cannibalistic gulls select eggs based on the absolute difference between the spot coverage of the egg and the most common spot coverage of eggs in the population.

METHODS

Data were collected at a Glaucous-winged Gull colony on Violet Point, Protection Island National Wildlife Refuge (48°07′40″N, 122°55′3″W), Washington State, located in the southeastern corner of the Strait of Juan de Fuca. Raw images of 718 individually marked gull eggs and data on the eventual fates of these eggs were collected during the 2015 breeding season. Raw images were made with a Canon 5D Mark II camera, mounted on a light stand within a darkened blind at the edge of the colony. Eggs were illuminated from two sides by Eiko DT36/50/RS, 36W CF, Long Twin Tube, 2G11 Base, 5000K fluorescent bulbs approximately 50 cm from the eggs. Each egg was accompanied by a UV-Vis-NIR reflectance standard reflecting 38.8% of light ranging from 250 to 2500 nm in wavelength. Photographed eggs were returned to their nests and monitored daily, with their fates determined according to methods described by Hayward et al. (2014).

Standardizing the Egg Image

I used ImageJ analysis software with a multispectral imaging calibration toolbox plugin to analyze egg images (Troschiano et al. 2015). The pixel values (i.e. gray values) of uncalibrated digital photographs were nonlinear with the amount of light measured by the sensor in the camera. Consequently, without some form of standardization, gray values from images could not be compared objectively. The multispectral imaging calibration toolbox used the image's gray standard in each photograph to control for lighting conditions so that pixel values could be compared objectively (Fig. 1). This ensured that gray values of each color channel were proportional to the brightness level of other colors. Details of how the egg image was standardized are given in the Appendix. I also created a region of interest (ROI) for each egg image. From this region, the program measured values such as the length, volume, and surface area of the egg in pixels. Then the egg ROI was selected, the outside was cleared, and the image was cropped. The image was renamed and saved to a library file.

Measuring Egg Coloration Data

The image created in the previous step was analyzed using the EggColorBrightness macro (created by Robert Polski, Department of Physics, Andrews University). The macro splits each pixel into its three channel components, red (R), green (G), and blue (B) (Fig. 2). After extracting each gray value, the macro combines each component back into its original photograph, and then takes an overall combined (C) value. Because the photographs analyzed were 32-bit RAW images, each of the three channels was an 8-bit image with a gray value that ranged from $0–2^8$. For each RGB channel, 0 represented the most intense color of that channel possible, represented by black, and 2^8 represented the least intense color, represented by white. Thus, each egg was characterized by its R, G, B, and C gray values.

Measuring Percent Spot Coverage

After characterizing the R, G, B, and C gray values for each egg image, the image was analyzed using the macro EggThresholdModeBackground (created by Robert Polski, Department of Physics, Andrews University). This macro first set the background and foreground color of each channel to the mode gray value of the channel. It then ran the image through a Fast Fourier Transform (FFT) bandpass filter. This filtered large structures down to 250 pixels, meaning that a region that contained more than 250 pixels would be smoothed out (blurred) by 5 pixels. The bandpass filter prevented isolated pixels that otherwise seemed like spots from being selected as spots, smoothed out structures, and ran the image through a MaxEntropy threshold (Fig. 3). The MaxEntropy threshold was used to select areas of the egg considered to be spots (Kapur et al. 1985). This was accomplished by calculating and comparing the entropy of neighboring areas to the spot of interest. ImageJ then returned the total area of spots selected on the ROI.

Data Analysis

Of the 718 egg images analyzed through ImageJ, I removed all those that were blurry or had an ImageJ threshold error, leaving 593 egg images for statistical analysis. Images without a clear distinction between the eggshell color and the background color were considered blurry images. Blurry images were removed because it was difficult to determine where the edge of the egg ended and where the black background began. An ImageJ threshold error occurred when the threshold occasionally over-selected regions of the image that were not part of the egg.

After ImageJ analysis, modal color values were determined using Excel and confirmed by plotting histograms (Fig. 4, Table 1). I used logistic regression to examine how the log-odds of egg fate were related to each of the following factors: gray value, absolute difference between gray value and the most common gray value (modal gray value), red value, green value, blue value, combined value, absolute difference between each color value (R, G, B, and C) and the modal color value, percent spot coverage, and absolute difference between spot coverage and the modal spot coverage. I used a separate regression analysis for each of these factors.

All statistical tests were carried out at the $\alpha = 0.1$ level.

RESULTS

Distributions of color values are shown in Fig. 4, and numerical modal color values are listed in Table 1. Log-odds of egg fate differences in relation to gray value, gray value distance from modal gray value, blue value, blue value distance from modal blue value, percent spot coverage, and percent spot coverage distance from modal percent spot coverage were not significant (Tables 2–5). Log-odds of egg fates in relation to red value and green value, respectively, were significant ($P = 0.0872$; $P = 0.0704$) (Table 4). The odds that an egg was cannibalized decreased by 1.6% with each increase of 10 red values. Similarly, the odds that an egg was cannibalized decreased by 1.5% with each increase of 10 green values.

Log-odds of egg fates in relation to green value distance from modal green value and combined value distance from modal combined value, respectively, were significant ($P = 0.089$; $P = 0.066$) (Table 5). The odds that an egg was cannibalized decreased by 19.3% with each additional 10 green values away from the modal green. Similarly, the odds that an egg was cannibalized decreased by 21.1% with each additional 10 combined values away from the modal combined value.

DISCUSSION

I showed that the odds of egg cannibalism are increased when an egg is more intensely red, more intensely green, when its green value is closer to the modal green value, and when its combined color value is closer to the modal combined color value. The modest levels of significance involved, however, indicate that more testing is warranted. There was no effect on the odds of cannibalism with respect to gray value, gray value distance from modal gray value, blue value, blue value distance from the modal blue value, percent spot coverage, and percent spot coverage distance from modal spot coverage.

An explanation for why the factors "distance from modal green" and "distance from modal combined" were significant may stem from the concept of search image. A search image describes the sensory recollection of an ideal prey item that a predator keeps in mind when seeking for it, and its formation is predicted to increase a predator's ability to detect such prey (Gendron et al. 1986). An egg cannibal may develop a search image for modally green and modally combined colored eggs, whose coloration would describe a stereotypical egg prey item. Nevertheless, the importance of the modal combined value is difficult to conceptualize. Combined values are calculated when ImageJ takes an average of red, green and blue values; consequently, I cannot determine whether a large combined value stems from a large red, green, or blue value, or from an overall increase in color intensity. Further research could confirm how modal combined values relate to modal coloration and search image explanations.

The significance of red and green intensity tentatively suggest that egg colors with longer wavelengths may play a role in egg selection by cannibals. In the electromagnetic spectrum, red and green have longer wavelengths than blue and ultraviolet light. Given that the odds of cannibalism increased when red value increased and when green value increased, cannibals may preferentially take eggs reflecting longer wavelengths. Reflectance of blue, which has a shorter wavelength, was not significantly associated with egg cannibalism. This is consistent with the results of Smith (2016), who showed that white chicken eggs, which reflected short wave UV light, were less likely to be cannibalized than UV-blocked eggs.

A study of camouflage might help explain the lack of significance for egg spot coverage factors in relation to cannibalism, given that spot pattern may contribute to an animal's ability to blend in with its surroundings. However, I did not collect data on the contrast in color between eggs and their nests or other aspects of camouflage. Using ImageJ methodology, Troscianko et al. (2016) evaluated images of the plumage of ground-nesting birds (plovers, coursers, and nightjars) and that of their surroundings. After documenting the fate of the birds and eggs, they discovered that the birds and their egg clutches were more likely to survive predation when the contrast between the eggs and their surroundings was low and when the plumage pattern of the birds closely matched their surroundings. Protection Island gulls also nest on the ground. Consequently, it would be interesting to apply the Troscianko et al. (2016) technique to the gull eggs and their nest backgrounds. Given the variety of nesting microhabitats and diversity of nest materials on the Protection Island colony, this would provide a valuable study.

In conclusion, I examined the relationship between Glaucous-winged Gull egg brightness, coloration, and spot coverage and the odds that an egg is cannibalized. The odds of egg cannibalism increased when an egg was more intensely red, more intensely green, and when its color was closer to the modal green value or closer to the modal combined value color. Research beyond the scope of this study may help determine why these particular factors were related to the incidence of cannibalism.

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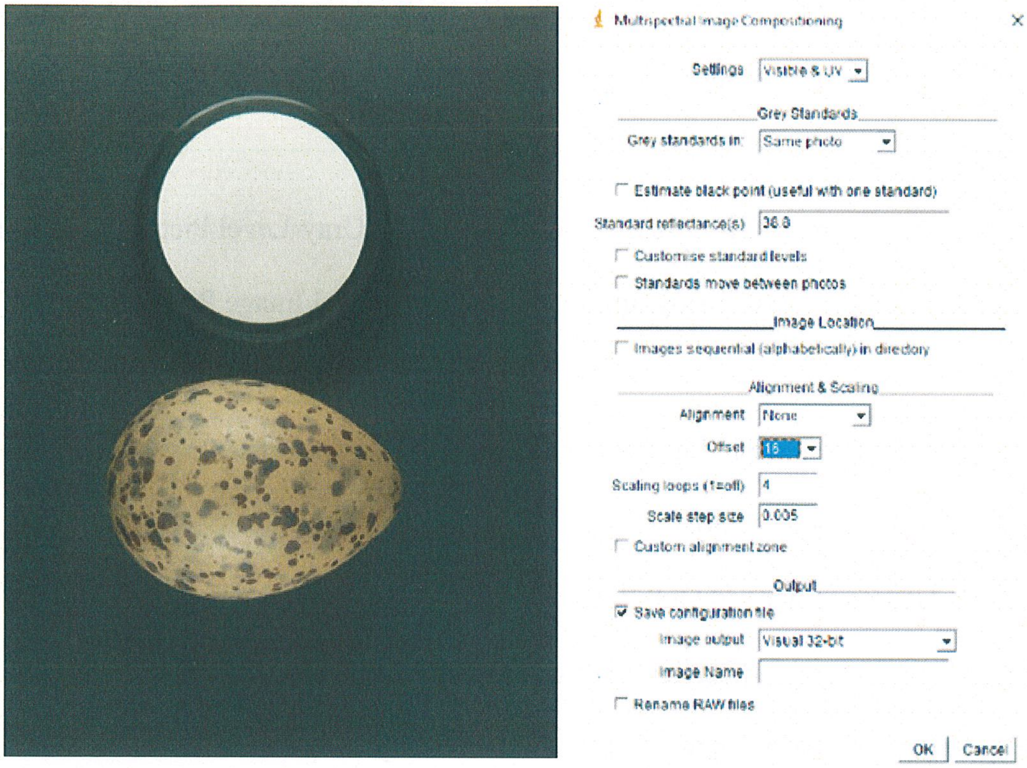

Figure 1. Example egg and multispectral imaging settings. The photograph on the right includes the UV-Vis-NIR 38.8% reflectance standard as well as the second (B) egg laid in nest 101. The image on the left is the window that appears when standardizing the egg image. Settings are set to Visible&UV, grey standards in same photo, no alignment, offset 16, scale size 0.005, and a 32-bit image output.

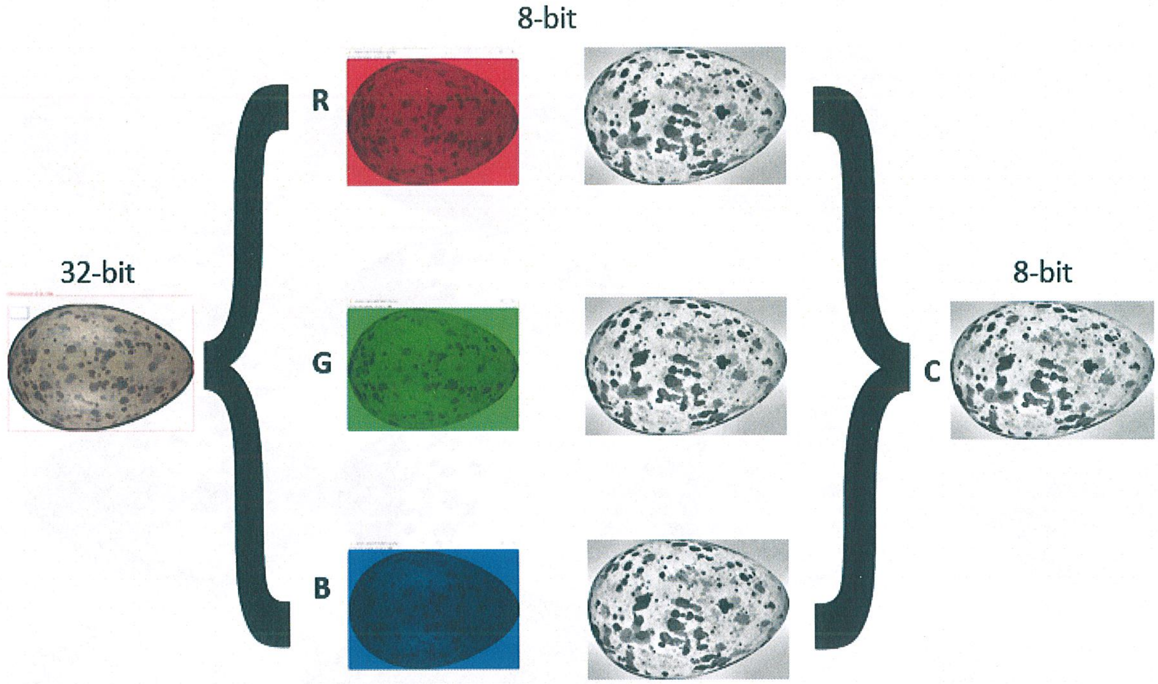

Figure 2. Representation of the functions of EggColorBrightness macro. The 32-bit image is split into red, green, and blue channels. After gray values are measured, they are combined into a single channel. No data are lost when splitting the 32-bit image into RGB channels, but data are lost when combining these channels into an 8-bit C image.

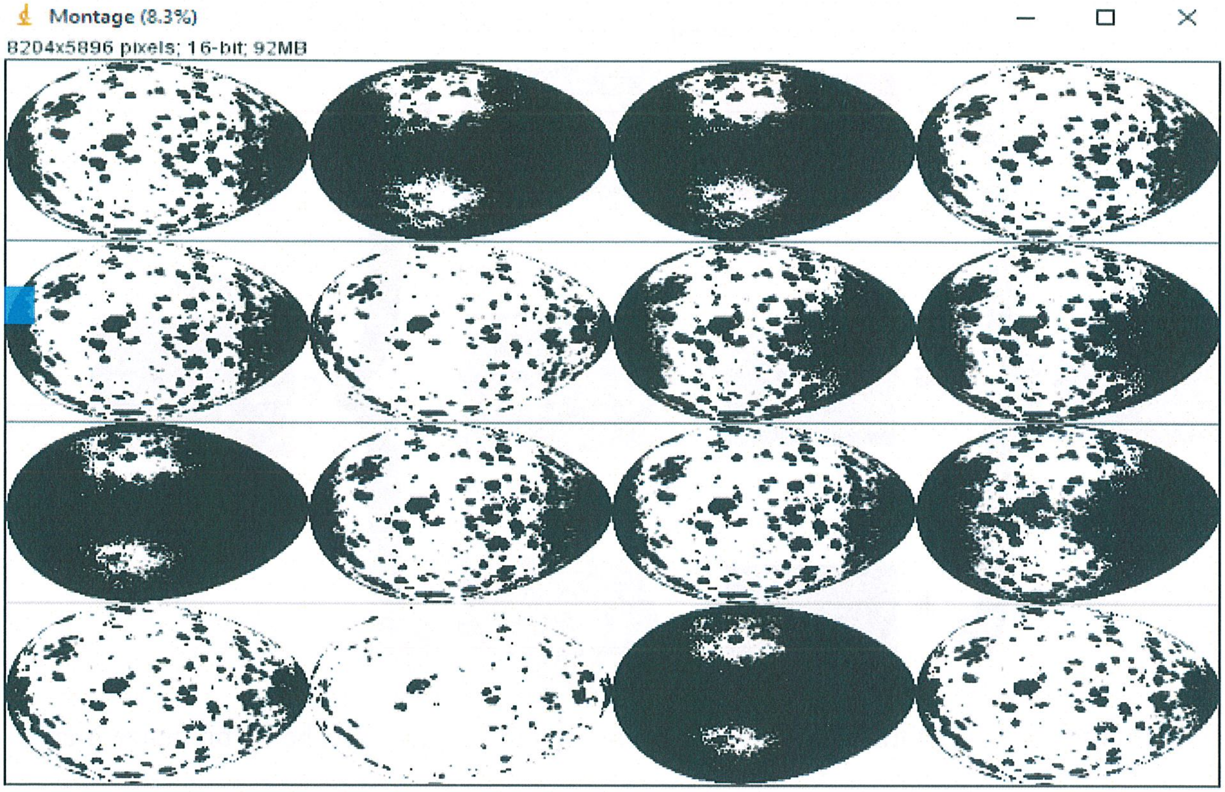

Figure 3. Different ImageJ auto-thresholding methods applied to the same egg. The egg from left to right and up to down were auto-thresholded with Default, Huang, Intermodes, IsoData, Li, MaxEntropy, Mean, MinError, Minimum, Moments, Otsu, Percentile, RenyiEntropy, ShanBhag, Triangle, and Yen. The egg image circled in red represents the result of MaxEntropy, the auto-thresholding method that was ultimately used to determine percent spot coverage. MaxEntropy was a thresholding method created by Kapur, *et. al.* (1985), which compares the entropy of a gray value of interest to the entropy of the gray values neighboring it.

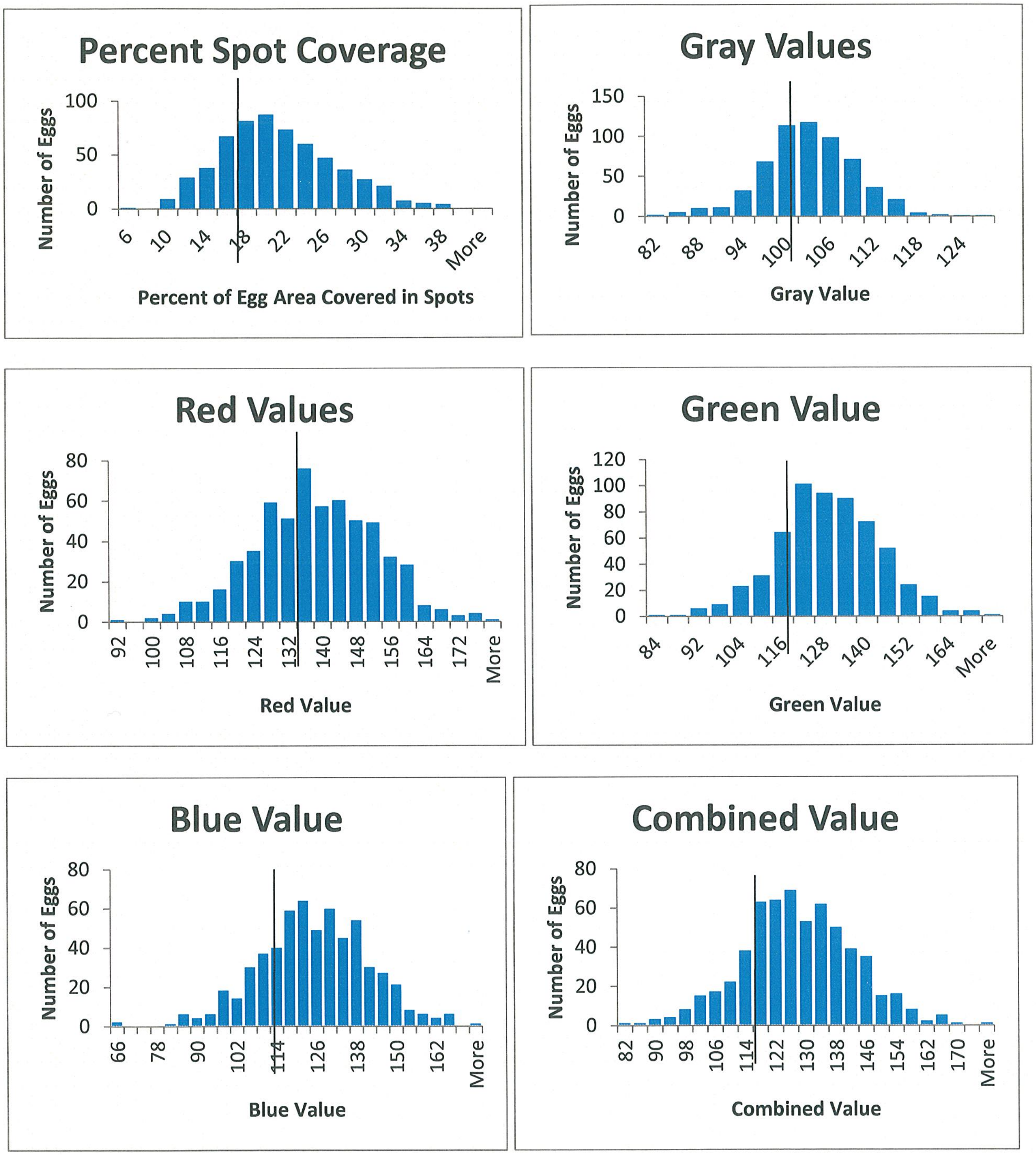

Figure 4. Histogram of the Percent Spot Coverage, Grey Value, Red, Green, Blue, and Combined of the 592 Photographed Gull Eggs. The black line represents the mode. Modal values are also given in α. The sample size was 593 data points.

Table 1. Modal Values for Grey Value, Red Value, Green Value, Blue Value, Combined Value, and Percent Spot Coverages. The sample size used was 593 data points.

Mode Grey	Mode Red	Mode Green	Mode Blue	Mode Combined	Mode Percent Spot Coverage
102	136	119	116	119	18

Table 2. Testing Subhypothesis A by regressing egg fate against gray value through logistic regression.

	Slope coefficient	P-value of the coefficient	Odds Ratio if C=5	Odds Ratio if C=10
Gray Value (GV)	-0.0039	0.838	0.981	0.962

Table 3. Testing Subhypothesis B by regressing egg fate against gray value distance from modal gray value using logistic regression.

	Slope coefficient	P-value	Odds Ratio if C=5	Odds Ratio if C=10
Gray Difference from Modal Gray	0.0131	0.654	1.0677	1.140

Table 4. Testing Subhypothesis C by regressing egg fate against red, green, blue, and combined values using logistic regression.

	Slope coefficient	P-value of coefficient	Odds Ratio if C=5	Odds Ratio if C=10
Red Value	-0.0016	0.0872	0.992	0.984
Green Value	-0.0015	0.0704	0.993	0.985
Blue Value	-0.0011	0.1791	0.995	0.989
Combined Value	-0.0017	0.652	0.992	0.983

Table 5. Testing Subhypothesis D by regressing egg fate against color distance from modal colors using logistic regression.

	Slope coefficient	P-value of coefficient	Odds Ratio if C=5	Odds Ratio if C=10
Red Difference from Modal Red	-0.0136	0.337	0.934	0.873
Green Difference from Modal Green	-0.0215	0.089	0.898	0.807
Blue Difference from Modal Blue	-0.0008	0.943	0.996	0.992
Combined Difference from Modal Combined	-0.0237	0.066	0.888	0.789

Table 6. Testing Subhypothesis E by regressing egg fate against percent spot coverage using logistic regression.

	Slope coefficient	P-value of coefficient	Odds Ratio if C=5	Odds Ratio if C=10
Percent Spot Coverage	0.0268	0.198	1.143	1.307

Table 7. Testing Subhypothesis F by regressing egg fate against percent spot coverage distance from modal percent spot coverage using logistic regression.

	b-coefficient	P-value	Odds Ratio if C=5	Odds Ratio if C=10
Percent Spot Coverage Difference from Modal Spot coverage	0.0445	0.0997	1.249	1.560

APPENDIX

Standardizing Egg Image

When I first received an uncalibrated egg image, I opened it through ImageJ>plugins>Multispectral Imaging>Generate Multispectral Image. See Fig. 1 for an uncalibrated egg image and the settings. When a dialogue window popped up and asked the user to select the reflectance standard, I selected the circular reflectance standard and clicked "OK". This standardized the pixel values for the entire image. Using the multi-point tool, I placed a point at the apex of the base, top, and each side of each egg. Between each of these regions, I added an additional 2–4 more points, finishing with a total of 12–20 points selected. Then I pressed "E". This selected an egg-like region connecting the points. If satisfied that the region selected matched the egg outline, "accept" was pressed. This created a region of interest (ROI) that appeared on the ROI window as "egg1". From there, the program measured values such as the length, volume, and surface area of the egg in pixels (px). Then "egg 1" ROI was selected, the outside was cleared, and the image was cropped. The image was renamed by nest number and order laid, and saved to a library file.